Rem fully inhibited [Ca^{2+}]-induced nuclear NFAT translocation and truncated Rem^{1-265} had no effect. Rem^{1-265}-Cav inhibited more than 80% of [Ca^{2+}]-induced NFAT nuclear translocation. **Conclusion**: These results suggest that $Ca_V1.2$ within Cav3 signaling microdomains is a major source of hypertrophic [Ca^{2+}] signaling and it can be blocked with little or no effect on excitation-contraction coupling.

3020-Pos Board B125
Dantrolene Restores Altered RyR2-Mediated Ca Signaling in Heart Failure
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In heart failure (HF) arrhythmogenic Ca release and chronic $[Ca]_{SR}$ depletion arise due to altered function of ryanodine receptors. Dantrolene, a therapeautic agent used to treat malignant hyperthermia associated with mutations of the type 1 ryanodine receptor (RyR1), is purported to be without effects on the cardiac type 2 ryanodine receptor (RyR2). However, recent investigations suggest that dantrolene may correct abnormal RyR2-mediated calcium release associated with HF. In this investigation, we tested if dantrolene exerts anti-arrhythmic effects on heart failure ventricular myocytes by examining the intra-SR Ca threshold for arrhythmogenic Ca waves. Using the low-affinity calcium indicator fluo-5N entrapped in the SR, direct measurement of $[Ca]_{SR}$ showed that in normal rabbit myocytes dantrolene (1 microM) had no effect on SR Ca content, the amplitude of action potential induced intra-SR Ca depletions, or on the threshold for spontaneous Ca wave initiation (i.e., the SR Ca content at which spontaneous waves initiate). Furthermore, in field stimulated (0.5 Hz and 1.0 Hz) normal cardiomyocytes loaded with indo-1, dantrolene treatment had no effect on Ca transient amplitude, SR Ca load, or post-rest decay of SR Ca content. In cardiomyocytes from failing rabbit hearts, SR Ca content and the wave initiation threshold were decreased compared to normal myocytes. Interestingly, treatment of HF cardiomyocytes with dantrolene restored the SR Ca content and increased the wave initiation threshold. Together, these data suggest that dantrolene may exert anti-arrhythmic effects in heart failure cardiomyocytes by increasing the intra-SR Ca threshold at which spontaneous Ca waves occur.

3021-Pos Board B126
A Stochastic Model of the Ryanodine Receptor Featuring Coupled Gating and Competitive Binding of Luminal and Cytosolic Ca^{2+} and Mg^{2+}
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During the last fifteen years, a number of computational models of local control of Ca2+ induced Ca2+ release have been presented. Recently, models for the activation of the ryanodine receptor (RyR), have been suggested which, include competitive binding of cytosolic and luminal Ca2+ and Mg2+. These models reproduce experimental spark frequency data obtained under different luminal and cytosolic Ca2+ and Mg2+ concentrations. However, they are steady-state models which cannot be used to study detailed spark kinetics, or they have only been used to fit RyR kinetics from bi-layer experiments. Here we present a stochastic and discrete model of the RyR featuring allosteric activation by competitive binding of luminal and cytosolic Ca2+ and Mg2+. The model also includes allosteric coupling between neighboring RyRs. We couple the model of the RyR with diffusional domains for both the junctional sarcoplasmic reticulumn and the dyadic cleft using a finite element model of diffusion. The allosteric coupling is modeled using a symmetric free energy approach, which keeps the number free parameters low. The model is fit to spark data from failing and SHAM-operated mice. The failing myocytes were aquired from a murine model of congestive heart failure (CHF). Myocardial infarction was induced by left coronary artery ligation, and at 10 weeks post-MI, mice exhibited symptoms of CHF. We use the computational model to explore the effect of phosphorylated RyRs in the failing myocytes.

3022-Pos Board B127
Determinants of the Site of Ca^{2+} Wave Initiation in Smooth Muscle
Marnie L. Olson, John G. McCarron.
Many smooth muscle activities including contraction, transcription and apoptosis are regulated by inositol 1,4,5-trisphosphate ($InsP_3$)-mediated increases in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_c$). Activation of surface receptors, such as muscarinic acetylcholine M3 receptors (mAChR3), leads to the production of $InsP_3$ to evoke Ca^{2+} release via receptors ($InsP_3R$) present on sarcoplasmic reticulum. Ca^{2+} release usually begins in a single 'eager' region and regeneratively propagates along the length of the cell as a Ca^{2+} wave. The Ca^{2+} wave repeatedly originates at the same 'eager' site. We addressed the mechanisms which determine the Ca^{2+} wave initiation site. One possibility is that the 'eager' site has a higher sensitivity to $InsP_3$ to evoke larger Ca^{2+} release. This does not appear to be the case because the site where waves initiated was not the site of largest Ca^{2+} release (as determined by local photolysis of caged $InsP_3$). The expression patterns of mAChR3 and $InsP_3R$ may provide an explanation. Although, there was no apparent regional receptor clustering, dual labelling of mAChR3 and $InsP_3R$ showed some receptor co-localization. Ca^{2+} wave initiation site may be determined by regions where the proximity of mAChR3 and $InsP_3R$ generate higher local [$InsP_3$] and $[Ca^{2+}]_c$. To explore this possibility the adaptability of the Ca^{2+} wave initiation site was examined by changing the local Ca^{2+} buffer capacity using the caged Ca^{2+} buffer diazo-2. Photolysis of diazo-2, at the site of wave initiation, during agonist application prevented initiation at this location. Yet, after a time lag the Ca^{2+} wave initiated at a new 'eager' site. These results indicate that the Ca^{2+} wave initiation site may be determined by co-localisation of mAChR3 and $InsP_3R$ and that the 'eager' site is altered when the $[Ca^{2+}]_c$ increase is prevented. Supported by the Wellcome Trust and British Heart Foundation.

3023-Pos Board B128
Sustainable TRPM4 Channel Activity Following Restoration of Cytosolic Calcium Buffering in Freshly Isolated Cerebral Smooth Muscle Cells
Albert L. Gonzales, Scott Earley.
The melastatin transient receptor potential (TRP) channel TRPM4 is a critical regulator of smooth muscle membrane potential and arterial tone. Activation of the channel is Ca^{2+}-dependent, but prolonged exposures to high intracellular Ca^{2+} causes rapid desensitization under conventional whole-cell patch clamp conditions. Using amphotericin B perforated whole-cell patch clamp electrophysiology which allows for minimal disruption of cytosolic Ca^{2+} dynamics, we recently showed that Ca^{2+} release from inositol trisphosphate receptors (IP_3R) activate TRPM4 channels, producing Transient Inward Cation Currents (TICCs). The coupling of IP_3R-mediated Ca^{2+}-release with activation of TRPM4 channels has not been fully characterized. We hypothesized that under conventional whole-cell conditions, loss of intrinsic cytosolic Ca^{2+} buffering following cell dialysis contributes to desensitization of TRPM4 channels. With the Ca^{2+} buffer ethylene glycol-bis(2-aminoethylether)-N,N,N′,N′-tetraacetic acid (EGTA, 10mM) included in the pipette solution, we were able to restore cytosolic Ca^{2+} buffering and record sustained TICC activity in freshly isolated cerebral smooth muscle cells. The total open probability for TICC activity was reduced following the administration of the TRPM4 inhibitor 9-phenanthrol and by siRNA-mediated knockdown of TRPM4, strongly suggesting that TICC activity is mediated through TRPM4. Lower concentrations of EGTA were not sufficient to restore TRPM4 activity. We further examined the spatial and temporal coupling between Ca^{2+} released through IP_3R and the activation of TRPM4 channels using the fast Ca^{2+} buffer bis-ethane-N,N,N',N'-tetraacetic acid (BAPTA) in the pipette. This study demonstrates our ability to restore Ca^{2+} buffering to physiological levels and allows for further examination of the coupling between IP_3R and TRPM4 activity in arterial smooth muscle cells RO1HL091905; F31HL094145-01.

Calcium Fluxes, Sparks, & Waves

3024-Pos Board B129
Modeling the Mechanisms of Calcium-Mediated Cardiac Arrhythmias
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Dysfunction of normal calcium dynamics has been implicated in the generation of cardiac arrhythmias. It is thought that spontaneous calcium release events in the myocyte lead to the formation of intracellular calcium waves. These calcium release events occur through opening of the ryanodine receptors (RyRs) in the sarcoplasmic reticulum. In order for this to lead to an arrhythmia, these waves need to depolarize the cardiac myocyte in events know as early afterdepolarizations (EADs) and delayed afterdepolarizations (DADs). These aberrant depolarizations must spread to adjacent cells in a propagating wave of depolarization to disrupt the normal pattern of electrical excitation of the heart. Computational compartmental ventricular myocyte models have shown that EADs and DADS can be generated by certain conditions consistent with experiments. We have developed a spatio-temporal computational model of a chain of cardiac myocytes based on the Jafri-Rice-Winslow model of the guinea pig ventricular myocyte. The model includes spatial resolution of the individual myocyte as well as a network of myocytes, calcium dynamics, and the sarcolemmal electrical activity. We use the model to explore how factors such as calcium overload, RyR calcium sensitivity, and other factors affect the generation of calcium waves. Furthermore, we also explore under what conditions the calcium wave can depolarize the myocyte and induce